Signaling Pathways and Potential Molecular Targets in Uveal Melanoma

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Abbreviations: UM: Uveal Melanoma

UVEAL MELANOMA

Uveal melanoma **(UM)** has an incidence rate varying from 5 to 10 per million people each year [1], accounting for 5-6% of primary systemic melanoma. The average age for presentation is around 60 years old. The occurrence rate inclines to be higher in male patients [2]. UM derives from uveal tract, which originates from the neural crest. Approximately 90% of UM develops from the choroid melanocytes, the remainder derives from the iris (3-5%) and the ciliary body (5-8%) [2]. Except for old age, the risk factors for UM include fair skin, light eyes, ocular melanocytosis and dysplastic nevus syndrome [3].

The diagnosis of UM mainly relies on clinical presentation, B scan ultrasound, fluorescent angiography, computerized tomography **(CT)** scan and magnetic resonance imaging **(MRI)**. Over 4% of micrometastasis is overlooked at the first time of diagnosis [4]. Current treatments such as surgery, brachy therapy and proton beam irradiation are effective in removal of focal tumor [5]. However, given the difficulty in diagnosis of early metastasis and tumor resistance to chemotherapy, systemic therapy is ineffective and the outcome for metastasis remains to be poor for decades. Although a more promising immune therapy has been brought into the systemic treatment of metastasized UM, the outcomes still await to be clarified by large-scale studies and long-term observations [4]. Almost half of the patients develop metastasis, 90% of the metastasis develop in the liver, which is the main cause of death [2]. Metastasis often leads to multifocal distribution of tumor lesions in the liver. Less than 9% liver involved patients develop isolate metastasis, enabling a surgical removal [4]. Once metastasis diseases occur, the one year survival rate is only 10-15% [6].

UM is featured in phenotypic plasticity, pathologically it can be divided into three phenotypes according to tumor cell morphology: spindle, mixed and epithelial UM. Spindle tumor cells are more alike their parental uveal melanocytes while epithelial tumor cells have dedifferentiated to lose their original morphology. The mixed tumor type contains both spindle and epithelioid tumor cells in the same tumor [7]. UM presented with more epithelial tumor cells usually show higher malignancy.

Generally, UMs with epithelioid morphology, loss of chromosome 3, involvement of the ciliary body, scleral invasion and BAP1 mutation are more inclined to develop metastasis [8,9]. The malignancy of UM lies in the biological features of tumor cells, which promote tumor growth, invasion, dedifferentiation and metastasis. Although the mechanism for UM tumorigenesis and distant dissemination is not completely clarified, reports found out that several signaling pathways are involved, therapies based on these molecular facts open new avenues for UM treatment. In this chapter, we will therefore focus on the molecular pathways underlying the mechanisms of UM development and the critical molecular targets in these pathways.

MOLECULAR PATHWAYS INVOLVED IN UM

Several signaling pathways regulate UM cell proliferation and survival. The phosphatidylinositol 3-kinase **(PI3K)** targets AKT to promote tumor angiogenesis and migration, leading to tumor growth and invasion [10]. The activated PI3K/AKT pathway can further activate mTOR that increases the expression of two downstream proteins 4E-BP1 and p70S6K, which in turn promotes the reconstruction of actin in filament, facilitating tumor cell movement [11-13]. The activation of MAPK pathway was found in UM independent of RAS and BRAF mutations, which is distinct from cutaneous melanoma **(CM)** [14]. PI3K/AKT cooperate with MAPK in antiapoptotic activities to elevate expression of vascular endothelial growth factor **(VEGF)**, tumor cell growth and maintenance [15,16]. Normally, TP53 is activated to promote transcription of cyclin-dependent kinase inhibitors **(CDKIs)** particularly P21CIP1 to activate RB1 to slow down cell cycle, to prevent

TP53 protein degradation and to transcript apoptosis-related genes like BAX leading to cell death [17]. In UM, RB1 was functionally inactivated as a result of cyclin/CDK complex phosphorylation that blocks its tumor suppressor activity [18]. The driver effectors of Hippo pathway YAP1 and TAZ trigger the transcription factors TEAD and SMAD, promoting tumor cell proliferation and inhibiting apoptosis [19]. G proteins activate YAP1/TAZ independent of Hippo and alternatively using a guanine nucleotide-exchange factor Trio and the downstream GTPases Rho and Rac in UM [20]. MMP2, MMP11 and PLAU are involved in UM cell migration and invasion [21,22]. BAP1 and MITF are associated with UM differentiation and development [22,23]. MEK-ERK signaling is activated through mutant GNAQ and GNA11 in UM. MEK inhibitor causes cell cycle arrest and apoptosis [24,25]. Hepatocyte growth factor **(HGF)** induces phosphorylation of its receptor MET, thereby triggers liver metastasis and induces resistance to MEK inhibitors in UM [26]. These various pathways provide multiple targets for UM therapies, some current or UM specific molecular targets are described in the following paragraphs.

BAP1

The loss of monosomy 3 contributes to UM poor prognosis and metastasis, studies sequenced the exon of the remaining monosomy 3 at the aim to find out UM pathogenesis-related crucial deoxy-nucleotide mutation. Breast cancer susceptibility gene 1 related protein 1 **(BAP1)** mutation was found in 85% metastasized UMs while hardly detected in non-metastasized tumors. BAP1 is a deubiquitinating enzyme which functions as a transcriptional repressor and inhibits tumor activity through suppressing tumor growth [27,28], modifying epigenetic phenotype [29], transcriptional regulation [30] and DNA damage responses [31,32]. BAP1 gene deletion is lethal during mice embryogenesis, knockout BAP1 in adult mice would recapitulate features of melanocytic tumors. In genetic modified mouse models, loss of BAP1 promotes cancer development including UM, malignant pleural mesothelioma [33], clear cell renal cell carcinoma [34] and cholangiocarcinoma.

UM can be stratified into two classes: Class 1 and Class 2 based on a commercially available gene expression profile **(GEP)** of 15 genes with distinct prognostic molecular signatures [35]. Recently PRAME gene as an independent biomarker, if combined with a 12-gene expression panel was also reported as a predictor for metastasis of UM [36]. Class 1UMs are more differentiated and have low propensity for metastasis, while Class 2 UMs are more dedifferentiated and exhibit a higher metastatic risk [37]. Prospective multicenter study furtherly confirmed the accuracy of the GEP in predicting metastasis and prognosis of Ums [35]. BAP1, EIF1AX, GNA11, GNAQ, and SF3B1 mutation are frequently found in tumors with Class 2 GEP [38]. BAP1 mutation was negatively correlated with SF3B1 mutation and EIF1AX mutation [39]. Germline mutation of BAP1 presents in 8% patients with metastatic UM [40], and is more frequently with early onset of UM [41]. Mutation of BAP1 in human UM might be a driving force for metastasis as levels of inactivation of BAP1 negatively correlated with the survival rates, and could serve as a predictor for poor prognosis. Immunohistochemistry has been recommended as a straightforward method for detecting the expression of BAP1 for prognosis of UM [42].

BAP1 exerts tumor suppressive activity through deubiquitinating and nuclear localization [43]. BAP1 interacts with the transcriptional regulator host cell factor 1 (HCF-1), whichrecruits H3K4 histone methyltransferases to the cell cycle S-phase gene activator E2F1 to inhibit its expression, leading toarrest of the cell cycle progression to the G1/S checkpoint [43,44]. Together with HCF-1, BAP1 controls the transcription factor Ying Yang1 **(YY1)** and regulates the transcription of many downstream genes [45]. Mutation of BAP1 is often present with certain kinds of DNA damages that provoke UM [46]. BAP1 also functions as a regulator for differentiation in uveal melanocyte, depletion of BAP1 would result in gain of stem cell properties such as expression of stem cell markers, self-replication potential and the ability to endure stem cell culture condition, although it is not shown that BAP1 associates with cell migration and tumorigenesis [23]. With ubiquitin binding and histone H2A deubiquitination, BAP1 is enabled to interact with sex comb-like protein ASXL2 to regulate cell senescence, disruption of BAP1/ASXL2 would induce tumor development [47]. Understanding how BAP1 regulates biological processes of UM would provide new target for UM treatment.

GNAQ AND GNA11

Over 80% of UM tumors harbor activating hotspot mutations in GNAQ or GNA11, which encodes alpha subunits of guanine nucleotide binding **(G)** proteins [48-50]. GNAQ and GNA11 mutationsmanifest early event of tumorigenesis in UM, intradermal melanocytic proliferations such as blue nevi with these mutations are predilect to develop UM [51]. Different from the commonly found BRAFV600E mutation in cutaneous melanoma, high expression of phosphorylated MEK and ERK proteins were found in UMs in the absence of BRAF mutations [14,52]. Mutations spot at residues 183 and 209 of the G proteins, result in the activation of MAPK and protein kinase C **(PKC)** pathways, with MAPK acting as the downstream of PKC activation [53]. The downstream MEK/ATK pathway induces activation of AMP-activated protein kinase in the GNAQ-mutant cells, inhibiting cell autophagy and apoptosis [54]. MEK1/2 inhibitor selumetinib, known as the inhibitor of MAPK pathway, reduces GNAQ mutant UM proliferation. GNAQ and GNA11 target on PKC and lead to ERK1/2 activation [55] and regulate cell cycle.

In mouse models, GNA11 Q209L and GNAQ 209L mutant melanocytes form primary tumors, GNAQ 209L mutant cells takes longer time in tumorigenesis. Mice injected with GNA11 mutant cells all developed metastases, whereas GNAQ mutant cells occasionally did not [51]. The Hippo tumor suppressive pathway is strongly regulated by GNAQ and GNA11 by triggering YAP/TAZ dephosphorylation and nuclear translocation [56]. YAP dephosphorylation and nuclear translocation further activates Notch signaling, which promotes UM cells viability and migration [57]. In UM cell lines, mutant GNAQ or GNA11 induces activation of YAP, the major oncogenic factor in Hippo pathway, leading to tumorigenesis [58]. In human UM samples, GNA11 mutations present in 67% metastatic lesion whereas GNAQ mutations present in 27% metastatic lesion [49]. Some studies reported that GNAQ and GNA11 mutations did not have significant association with the risk of metastasis [8,59]. The mutation in GNAQ or GNA11 also induces influx of macrophage,

which is associated with neovascularization,thereby may accounts for the enriched blood supply in UM [60].

EMT RELATED TRANSCRIPTION FACTORS

EMT is a process for epithelial cells to transdifferentiate to motile mesenchymal phenotype, which functions crucially in embryogenesis, fibrosis, tumorigenesis and metastasis, along with cancer therapy resistance [61,62]. In epithelium tumors, cancer cells obtain cell plasticity and mesenchymal morphology through EMT. The reverse process of EMT, also known as MET, facilitates metastatic colonization in distant sites by disseminated tumor cells. More importantly, EMT results in acquisition of cancer stem cell like features such as self-renewal and lower proliferation rate, leading to cancer therapy resistance, recurrence and poor prognosis [63].

Both EMT and MET are driven by a group of EMT transcription factors **(TFs)** including TWIST, SNAI and ZEB families. In carcinomas, EMT-TFs contribute to accelerate EMT progress. However, for solid tumors other than carcinomas such as UM, the molecular mechanisms through which EMT-TFs contribute to their malignant progression have not been well elucidated. New evidence shows that EMT is dispensable for metastasis [64,65] and that all EMT inducers are not equal in cell transformation [66]. Among these EMT-TFs, ZEB1 facilitates tumorigenesis and invasion by inducing EMT, as well as enhancing tumor radio resistance [66,67].

Researchers have identified ZEB1 as a major player during the cadherin switch in melanoma [68], certain tumor microenvironmental factors such as *TGFβ* and hypoxia regulate ZEB1 during melanoma phenotype switching and repress MITF expression, eventually leading to melanoma invasion and metastasis [69-71]. Recently, researchers have also reported that down-regulation of ZEB1, TWIST1 and SNAI1 reduces the invasive properties of UM cells. ZEB1 binds to MMP11 and PLAU, two genes controlling tumor cell invasion and migration; MITF and BAP1, which are involved in UM pigment synthesis and differentiation; CDKN1A, which is crucial for rapid cell proliferation; ABCB1, which has a role in chemo-resistance and stem cell property. Repressing ZEB1 result in reduced ability in tumor cell invasiveness, migration, proliferation, and dedifferentiation, independent from EMT process.

EMT process that generally accounts for phenotype transition in epithelium tumors does not necessarily involved in UM. However, the EMT-TFs still have significant roles in controlling UM malignancy [72,73]. Among these EMT-TFs, ZEB1 functions in determining malignant properties. Dysregulation of ZEB1 expression governs UM proliferation, invasion, migration, differentiation and metastasis. More importantly, gene expression profiling of two large cohorts of human primary UMs clearly shows that high expression of ZEB1 significantly predisposes the tumor to metastasis, suggesting that ZEB1 could be used as a oncogenic factor denoting poor prognosis in UM [22]. Novel therapies targeting ZEB1 would be promising with a more comprehensive understanding of ZEB1 dysregulation and the associated cellular signaling in UM.

HGF

UMs have a tendency to metastasize to the liver and most of them are highly resistant to targeted therapies such as MEK inhibitors. Efforts have been made to determine whether UM cells and hepatic cells have similar microenvironment. Hepatocyte growth factor **(HGF)** is secreted by quiescent hepatic stellate cells and its corresponding receptor is tyrosine kinase receptor MET [25]. The expression of MET is higher in metastasized lesions than in primary UMs and associated with UM mortality [74]. Study of 132 human primary UM specimen suggested that HGF may play a crucial role in spreading of UM to the liver [75]. Primary hepatic stellate cell medium protects UM cells against MEK inhibitor trametinib.HGF provides resistance to MEK inhibitors, which interrupt cell cycle arrest and the procession of apoptosis [74]. Activated HGF increases expression of MET, which is correlated with the invasive and migration ability in UM cell lines [76]. Target of HGF and its receptor MET could be a potential effective method for chemo-resistant liver metastasized UM.

MMPS

Matrix metalloproteinases **(MMPs)** are a group of calcium-dependent zinc-containing endopeptidases, have the ability to degrade basement membranes in the extracellular matrix as well as boosting vascular density, tumor growth, cellular invasion and distant metastasis [77,78]. MMPs are generally classified into collagenases (MMP-1, -8 and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3 and -10), matrilysins (MMP-7, -11 and -26), membrane-type MMPs (MMP-14, -15, -16, -17, -24 and -25) and other MMPs (MMP-12, -19, -20, -21, -23, -27 and -28) [79]. MMP9 was reported to predominantly present in the more aggressive epithelioid UM or the epithelial region of mixed type of UM, with poor outcome of UM patients [80]. Anti-vascular growth therapies could result in compensatory increase of MMP9 secretion, enabling UM cells to bypass the anti-VEGF inhibition [81]. Increased expression of MMP2 was found in different UM cell lines and associated with UM progression [82], its relevance to prognosis is controversial [83,84]. The inhibition of ERK1/2 could downregulate the secretion of MMP2 thereby reduce the UM migration activity [85]. The tissue inhibitors of metalloproteinases **(TIMPs)** can inhibit the physiological activities of MMPs.TIMP1 and 2 suppress MMP2 and 9 activities respectively, human tumor specimen expressing TIMP1 and 2 had better survival rate [86]. TIMP3 is associated with better prognosis of UM [87]. In human UM specimens, all UM tumors express strong MMP-2, as well as heterogeneous expression of MMP-9 and weak TIMP-2 on tumor vasculature, indicating their role in tumor growth and angiogenesis [88].

CONCLUSION

UM is a malignant cancer that leads to metastatic death in up to half of patients. It is initially asymptomatic, and micrometastasis usually already exists at the time of diagnosis, which accounts for the high death rate for UM [89]. Early diagnosis and systemic therapy remain as a challenge because the pathogenic mechanism of tumor progression of UM is not clear [1]. Dysregulation of various signaling pathways and mutations of multiple genes promotes UM proliferation, invasion

and metastasis. Although the regulation of UM tumorigenesis is complex, some of the genes share common pathways in tumor development. Targeting the crucial molecule in these pathways may provide effective intervention in UM therapy. A more comprehensive understanding of molecular dysregulation and the associated abnormalities in cellular signaling in UM would enable the emergence of more specific and targeted novel therapies.

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